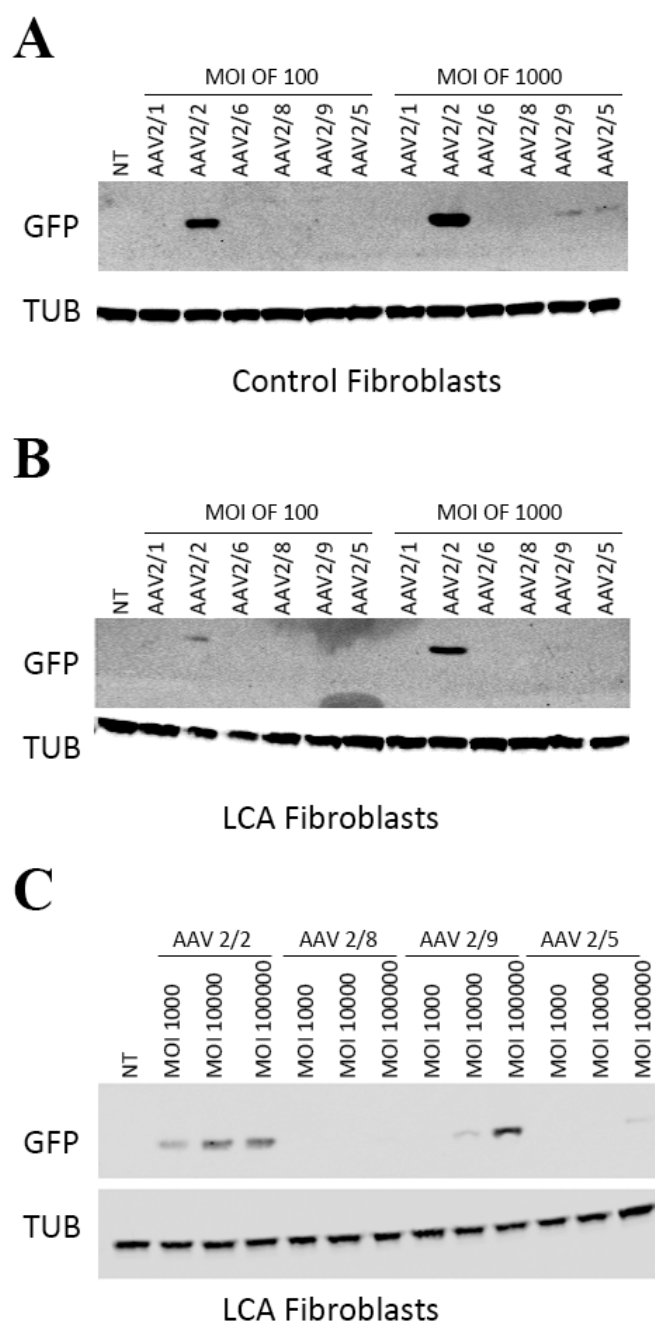


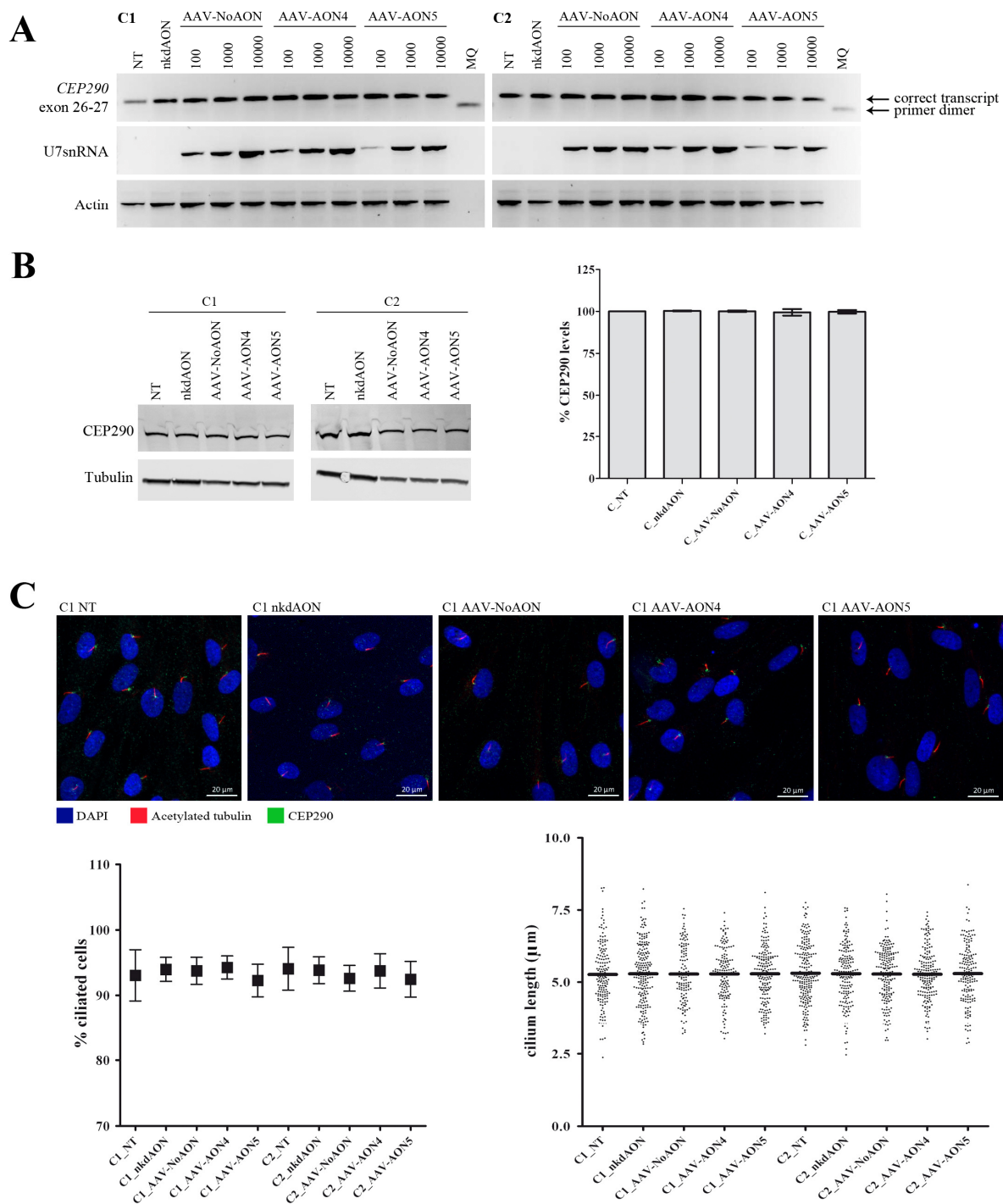
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Transduction efficacy of various GFP-expressing AAV serotypes

A-B) Western blot analysis of control (**A**) and LCA patient (**B**) fibroblast cells transduced with different AAV serotypes, each containing the same GFP expression cassette under control of a CMV promoter. Cells were transduced at two different MOIs.

C) Further analysis in LCA fibroblasts using higher MOIs for AAV2/2, AAV2/5, AAV2/8 and AAV2/9. AAV2/2 showed the best transduction efficiency. Tubulin was used in all cases as a loading control.

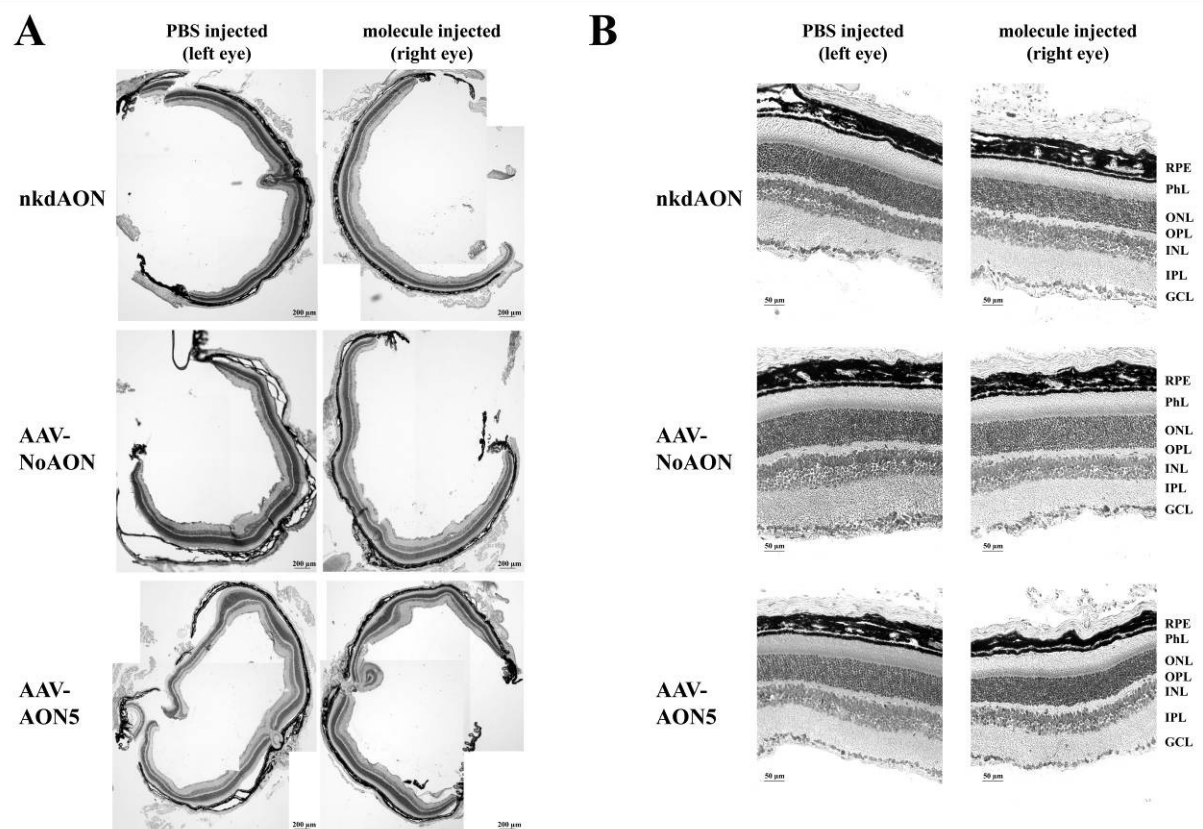


Supplementary Figure 2. Assessment of AAV-AON treatment in control cell lines

A) RT-PCR analysis on control fibroblast cells treated with AAV-NoAON, AAV-AON4 and AAV-AON5 at three different MOI (100; 1,000 and 10,000). No differences were observed between treated and untreated cells. U7snRNA was used as a transduction control and actin as a loading control.

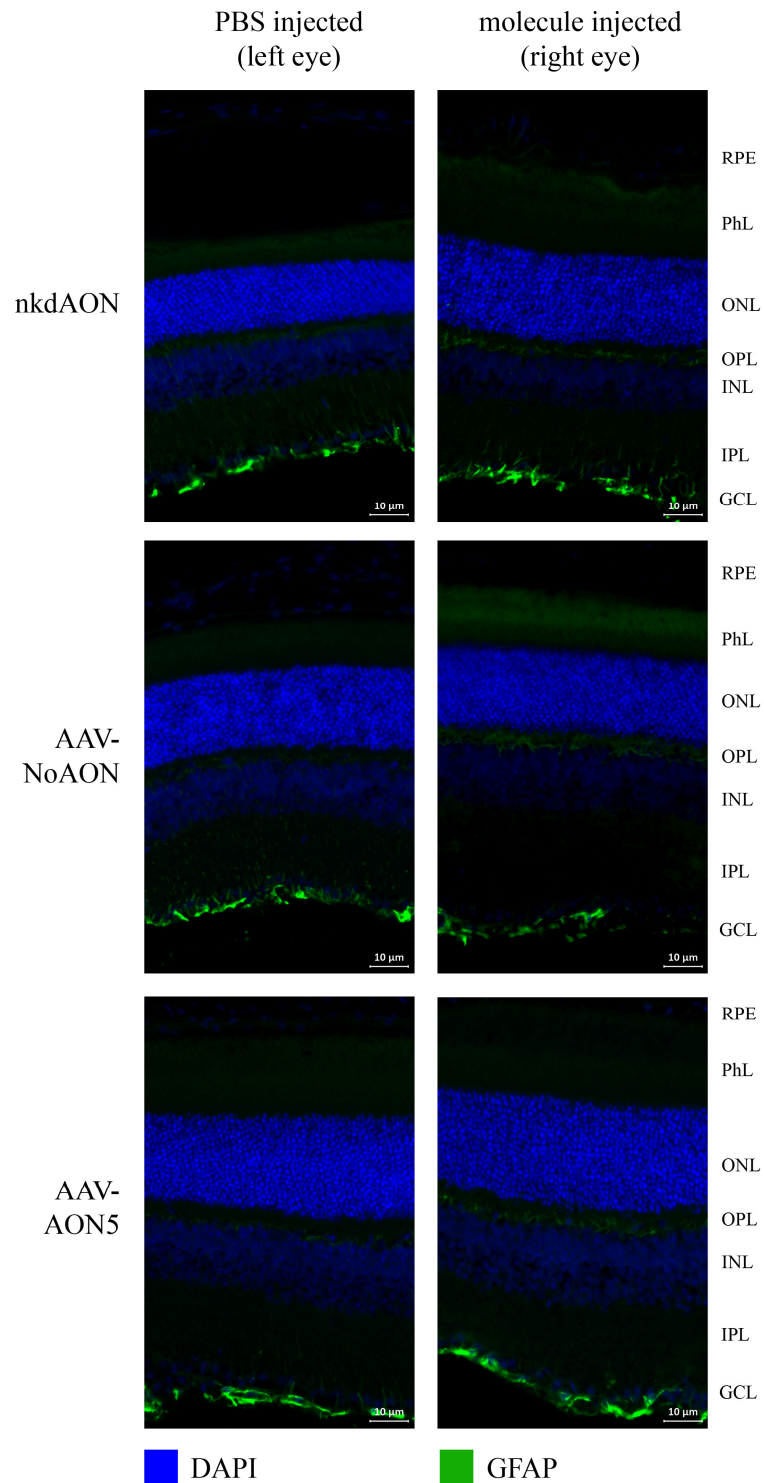
B) Western blot analysis of CEP290 protein levels on control fibroblast cells transduced with AAV-NoAON, AAV-AON4 and AAV-AON5. Tubulin was used as a loading control for normalization. No differences were observed between treated and untreated cells.

C) Immunocytochemistry on control fibroblast cells treated with the three AAVs. CEP290 is shown in green and localizes to the basal body in all conditions. The cilium axoneme was stained with acetylated tubulin in red and nuclei (DAPI staining) are presented in blue. The percentage of ciliated cells as well as the average cilium length did not differ between treated and non-treated (NT) cells.



Supplementary Figure 3. Assessment of the structure of the retina after treatment

Seven micrometer cryosections stained with toluidine blue. **A)** 50X magnification images covering the complete retina. **B)** 400X magnification images. RPE: Retinal Pigment Epithelium; PhL: Photoreceptor Layer; ONL: Outer Nuclear Layer; OPL: Outer Plexiform layer; INL: Inner Nuclear Layer; IPL: Inner Plexiform Layer and GCL: Ganglion Cell Layer.



Supplementary Figure 4. GFAP immunostaining

Immunostaining of 7 μm cryosections from mice treated with PBS, naked AON, AAV-NoAON or AAV-AON5. DAPI (blue) stains the nuclei while GFAP (green) is an indicator of gliosis and structural stress in the retina. No differences were observed. RPE: Retinal Pigment Epithelium; PhL: Photoreceptor Layer; ONL: Outer Nuclear Layer; OPL: Outer Plexiform layer; INL: Inner Nuclear Layer; IPL: Inner Plexiform Layer and GCL: Ganglion Cell Layer.

SUPPLEMENTARY TABLE

Supplementary Table 1 - Oligonucleotide sequences

RT-PCR			
Forward primers		Reverse primers	
Name	Sequence (5'→3')	Name	Sequence (5'→3')
CEP290ex26	TGCTAAGTACAGGGACATCTTGC	CEP290ex27	AGACTCCACTTGTTCTTTTAAGGAG
U7snRNA	GGGTCTAGATAACAACATAGGAGCTGTGA	U7snRNA	AAAGCTAGCCACAACGCGTTTCCTAGGA
Rhodopsin	ATCTGCTGCGGCAAGAAC	Rhodopsin	AGGTGTAGGGGATGGGAGAC
Actin	ACTGGGACGACATGGAGAAG	Actin	TCTCAGCTGTGGTGGTGAAG
		CEP290exX	CAACTGGGGCCAGGTGC
Cloning pAAV-AON			
Forward primers		Reverse primers	
Name	Sequence (5'→3')	Name	Sequence (5'→3')
NoAON	CCGCAAAATTTTGGAGCAGGTTTCTGAC	NoAON	AAAATTTTGCGGAAGTGCGTCTGTAG
AON1	AACTGGGGCCAGGTGCGAATTTTGGAGCAGGTTTCTGAC	AON1	CGCACCTGGCCCCAGTTTTCGGAAGTGCGTCTGTAG
AON2	CACATCGTAATCCAGCACTTTAGGAGGAATTTTGGAGCAGGTTTCTGAC	AON2	GATTACGATGTGAGCCACCGCACCTGGTTGCGGAAGTGCGTCTGTAG
AON3	TGGGGGTAATCCAGCACTTTAGGAGGAATTTTGGAGCAGGTTTCTGAC	AON3	GATTACCCCCAGTTGTAATTGTGAGTATCTTGCGGAAGTGCGTCTGTAG
AON4	GTGCGGTGGCTCACATCAATTTTGGAGCAGGTTTCTGAC	AON4	TGAGCCACCGCACCTGGTTGCGGAAGTGCGTCTGTAG
AON5	TGGGGCCAGGTGCGGTGGCTCACATCAATTTTGGAGCAGGTTTCTGAC	AON5	CCTGGCCCCAGTTGTAATTGTGAGTATCTTGCGGAAGTGCGTCTGTAG
AON6	GGGGCCAGGTGCGGTGGAATTTTGGAGCAGGTTTCTGAC	AON6	CACCTGGCCCCAGTTGTATTGCGGAAGTGCGTCTGTAG